Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney

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Edited by Richard P. Lifton, Yale University School of Medicine, New Haven, CT, and approved September 27, 2006 (received for review July 3, 2006)

Essential hypertension is a common disease, yet its pathogenesis is not well understood. Altered control of sodium excretion in the kidney may be a key causative feature, but this has been difficult to test experimentally, and recent studies have challenged this hypothesis. Based on the critical role of the renin-angiotensin system (RAS) and the type I (AT_1) angiotensin receptor in essential hypertension, we developed an experimental model to separate AT_1 receptor pools in the kidney from those in all other tissues. Although actions of the RAS in a variety of target organs have the potential to promote high blood pressure and end-organ damage, we show here that angiotensin II causes hypertension primarily through effects on AT_1 receptors in the kidney. We find that renal AT_1 receptors are absolutely required for the development of angiotensin II-dependent hypertension and cardiac hypertrophy. When AT_1 receptors are eliminated from the kidney, the residual repertoire of systemic, extrarenal AT_1 receptors is not sufficient to induce hypertension or cardiac hypertrophy. Our findings demonstrate the critical role of the kidney in the pathogenesis of hypertension and its cardiovascular complications. Further, they suggest that the major mechanism of action of RAS inhibitors in hypertension is attenuation of angiotensin II effects in the kidney.

transgenic mice | kidney transplantation | blood pressure

High blood pressure (BP) is a highly prevalent disorder, and its complications (including heart disease, stroke, and kidney disease) are a major public health problem (1). Despite decades of scrutiny, the precise pathogenesis of essential hypertension has been difficult to delineate. Guyton and his associates suggested that defective handling of sodium by the kidney and consequent dysregulation of body fluid volumes is a requisite, final common pathway in hypertension pathogenesis (2). The powerful capacity of this pathway to modulate blood pressure is illustrated by the elegant studies of Lifton and associates showing that virtually all of the Mendelian disorders with major impact on blood pressure homeostasis are caused by genetic variants affecting salt and water reabsorption by the distal nephron (3). On the other hand, several recent studies have suggested that primary vascular defects may cause hypertension by impacting peripheral resistance without direct involvement of renal excretory functions (4–7).

Among the various regulatory systems that impact blood pressure, the RAS has a key role. Inappropriate activation of the RAS, as in renal artery stenosis, leads to profound hypertension and cardiovascular morbidity (8). Moreover, in patients with essential hypertension who typically lack overt signs of RAS activation, ACE inhibitors and angiotensin receptor blockers (ARBs) effectively reduce blood pressure and ameliorate cardiovascular complications (9–11), suggesting that dysregulation of the RAS contributes to their elevated blood pressure.

At the cellular level, responsiveness to angiotensin II (Ang II) is conferred by the expression of the two classes of angiotensin receptors (AT_1 and AT_2). The effects of Ang II to increase blood pressure are mediated by AT_1 receptors (12), and these receptors are expressed in a variety of organ systems thought to play key roles in blood pressure homeostasis, including the heart, kidney, blood vessels, adrenal glands, and cardiovascular control centers in the brain (13). For example, in the vascular system, stimulation of AT_1 receptors causes potent vasoconstriction (14, 15). In the adrenal cortex, their activation stimulates the release of aldosterone (16) that in turn promotes sodium reabsorption in the mineralocorticoid-responsive segments of the distal nephron (17). In the brain, intraventricular injection of Ang II causes a dramatic pressor response that is mediated by AT_{1A} receptors (18). In the kidney, activation of AT_1 receptors is associated with renal vasoconstriction and antinatriuresis (19, 20). Nevertheless, whether angiotensin actions in these individual tissue sites contribute *in vivo* to the pathogenesis of hypertension and its complications is not clear.

To address this question, we used a kidney cross-transplantation strategy to separate the actions of AT_1 receptor pools in the kidney from those in systemic tissues. Our findings suggest that AT_1 receptors expressed in the kidney are the primary determinants of hypertension and end-organ damage in Ang II-dependent hypertension.

Results

Kidney Cross-Transplantation Model. We used a kidney cross-transplantation strategy to separate the actions of AT_1 receptor pools in the kidney from those in systemic tissues, as we have described previously (21). Kidney transplantation was carried out between genetically matched F_1(C57BL/6 × 129) wild-type mice and F_1(C57BL/6 × 129) mice homozygous for a targeted disruption of the *Agtr1a* gene locus encoding the AT_{1A} receptor (14). The AT_{1A} receptor is the major AT_1 receptor isoform in the mouse and the closest mouse homologue to the human AT_1 receptor gene (12).

By varying the genotype of the transplant donor and recipient, we generated four groups of animals in which renal function was provided entirely by the single transplanted kidney. The *Wild-type* group consisted of wild-type mice transplanted with kidneys from wild-type donors and thus have normal expression of AT_{1A} receptors in the kidney transplant and in all systemic tissues. For the *Systemic KO* group, AT_{1A} receptor-deficient recipients were transplanted with kidneys from wild-type donors; these animals lack AT_{1A} receptors in all tissues *except* the kidney. *Kidney KO* animals are wild-type recipients of AT_{1A} receptor-deficient kidneys, thus lacking expression of AT_{1A} receptors only in the kidney, but with normal expression of receptors in all systemic, nonrenal tissues, including the adrenal gland. Finally, the *Total KO* group consists of AT_{1A} receptor-deficient recipients of AT_{1A} receptor-deficient kidneys and therefore completely lacking AT_{1A} receptors in all tissues.

Author contributions: S.D.C., O.S., T.H.L., and T.M.C. designed research; S.D.C., S.B.G., M.J.H., P.R., R.G., A.P.K., and H.-S.K. performed research; S.D.C., S.B.G., P.R., T.H.L., and T.M.C. analyzed data; and S.D.C., O.S., T.H.L., and T.M.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS direct submission.

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Baseline Blood Pressure Measurements. One week after the transplantation procedure, radiotelemetry transmitters were implanted to provide direct measurements of arterial pressures in the mice in a conscious and unrestrained state. One week after placement of these units, when the animals had regained normal diurnal variation of blood pressure, blood pressure measurement was initiated. Baseline blood pressures in the *Systemic KO* and *Kidney KO* groups were virtually identical [109 ± 1 mmHg vs. 109 ± 1 mmHg (1 mmHg = 133 Pa)] and intermediate to those of the *Wild-type* (114 ± 2 mmHg) and *Total KO* (97 ± 2 mmHg) groups, consistent with our previous experiments (21) showing that renal and systemic AT_1 receptors make equivalent contributions to the level of blood pressure in the basal state.

A Major Role for AT_1 Receptors in the Kidney in Ang II-Dependent Hypertension. To distinguish the AT_1 receptor population that is critical for the pathogenesis of hypertension, osmotic minipumps were implanted s.c. into each animal to infuse Ang II (1,000 ng/kg/min) continuously for 4 weeks. This is a widely used model of experimental hypertension in which elevated blood pressure is mediated by ligand stimulation of AT_1 receptors causing significant end-organ damage, including cardiac hypertrophy (22–24). Upon initiation of Ang II infusion, mean arterial pressures (MAP) in the *Wild-type* transplant group rose dramatically to almost 160 mmHg (Fig. 1*A*) and remained elevated throughout the infusion period (MAP of 166 ± 3 mmHg for Week 3 of infusion; mean BP increase of + 55 ± 3 mmHg). This degree of blood pressure increase is similar to that seen in previous studies using nontransplanted mice (23), suggesting that the transplant procedure and the presence of only a single kidney does not significantly alter blood pressure responses to chronic Ang II infusion. By contrast, blood pressures in the *Total KO* animals that are completely devoid of AT_{1A} receptors were affected only minimally by Ang II infusion (Fig. 1*A*), reflecting the key role of AT_1 receptors in the development of hypertension in this model (MAP of 104 ± 3 mmHg at Week 3). The modest (+ 6 ± 3 mmHg; $P = 0.05$) increase in blood pressure in these animals was likely mediated by expression of the minor AT_1 receptor isoform, AT_{1B}, which is unaffected by the AT_{1A} gene disruption (15).

The *Kidney KO* animals (Fig. 1*A*) experienced an immediate increase in blood pressure when the Ang II infusion was initiated, peaking on Day 2 (135 ± 5 mmHg; $P < 0.0003$ vs. *Kidney KO* baseline) and rapidly receding thereafter. However, at every time point, including Day 2, blood pressures in the *Kidney KOs* were significantly lower than those in the *Wild-type* group. Accordingly, the degree of hypertension was markedly attenuated in the *Kidney KOs* compared with the *Wild-type* group (MAP of 126 ± 5 vs. 166 ± 3 mmHg at Week 3; $P = 0.0001$). Moreover, the extent of the blood pressure increase in the *Kidney KO* group was not different from that seen in the *Total KOs* (+ 15 ± 4 mmHg; P = NS vs. *Total KO*). Despite the early and transient increase in blood pressure, presumably due to peripheral vasoconstriction, the absence of AT_{1A} receptors in the kidney alone is sufficient to protect from Ang II-dependent hypertension.

In contrast, in the *Systemic KO* animals expressing AT_{1A} receptors *only* in the kidney (Fig. 1*A*), MAP rose progressively over the first 2 weeks of Ang II infusion. Yet, their blood pressures lagged behind the higher pressures seen in the *Wild-type* group for the first 10 days, likely reflecting the transient contribution of the early vasoconstrictor response seen in the *Kidney KOs*. Nonetheless, from Day 4 onward, blood pressures in *Systemic KOs* significantly exceeded those of the *Kidney KOs* ($P < 0.008$). Furthermore, by Day 12, blood pressures in the *Systemic KOs* converged with and were virtually identical to those of the *Wild-type* group (161 ± 5 mmHg for Week 3; mean BP increase of + 51 ± 4 mmHg; P = NS vs. *Wild-type*; $P = 0.0003$ vs. *Kidney KO*). Accordingly, the presence of AT_{1A} receptors in the kidney alone is sufficient to recapitulate the hypertension phenotype of the *Wild-type* group. Together, these data show that Ang II causes hypertension primarily through AT_{1A} receptors expressed in the kidney.

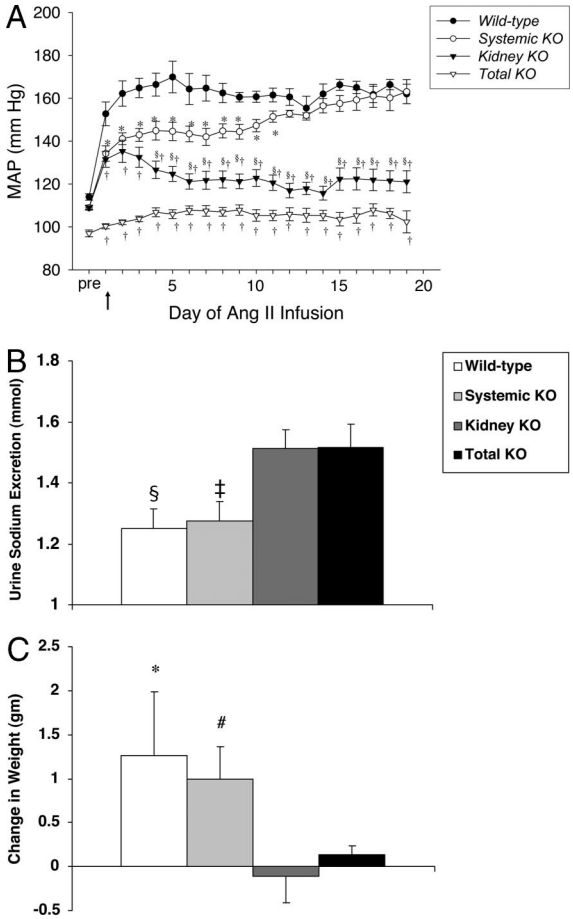

Fig. 1. Blood pressures and urinary sodium excretion in mice after kidney cross-transplantation. (*A*) Daily, 24-h blood pressures in the experimental groups before (''pre'') and during 21 days of Ang II infusion (*, $P \leq 0.03$ vs. *Wild-type*; §, $P < 0.008$ vs. *Systemic KO*; †, $P < 0.006$–0.0001 vs. *Wild-type*). (*B*) Cumulative sodium excretion during the first 5 days of Ang II infusion. (§, $P < 0.02$ vs. *Kidney KO* and $P = 0.03$ vs. *Total KO*; ‡, $P = 0.03$ vs. *Kidney KO* and *Total KO*). (*C*) Change in body weights after 5 days of Ang II infusion. (*, $P = 0.03$ vs. ''pre''; #, $P = 0.05$ vs. ''pre'').

Urinary Sodium Excretion. We reasoned that activation of AT_1 receptors in the kidney might cause hypertension by influencing renal sodium handling (2). Therefore, we compared urinary sodium excretion between the experimental groups during the first week of Ang II infusion. The animals were placed in metabolic cages (25), and food and water intakes were matched to avoid confounding effects of variable intake on urinary electrolyte excretion. Before implantation of Ang II-infusion pumps, total urinary sodium excretion ($U_{Na}V$) was similar in the four experimental groups (not shown). In contrast, cumulative sodium excretion measured during the first 5 days of Ang II infusion (Fig. 1*B*) was significantly reduced in the hypertensive *Wild-type* and *Systemic KO* groups compared with the *Kidney KO* and *Total KO* groups. The latter two groups lack AT_{1A} receptors in the kidney and are resistant to the hypertensive actions of Ang II (Fig. 1*A*).

Impaired sodium excretion by the kidney may increase blood pressure by expanding intravascular fluid volume (2), which should be reflected acutely by changes in body weight. Therefore, to determine whether the reduced sodium excretion in the *Wild-type* and *Systemic KO* groups led to volume expansion, we compared

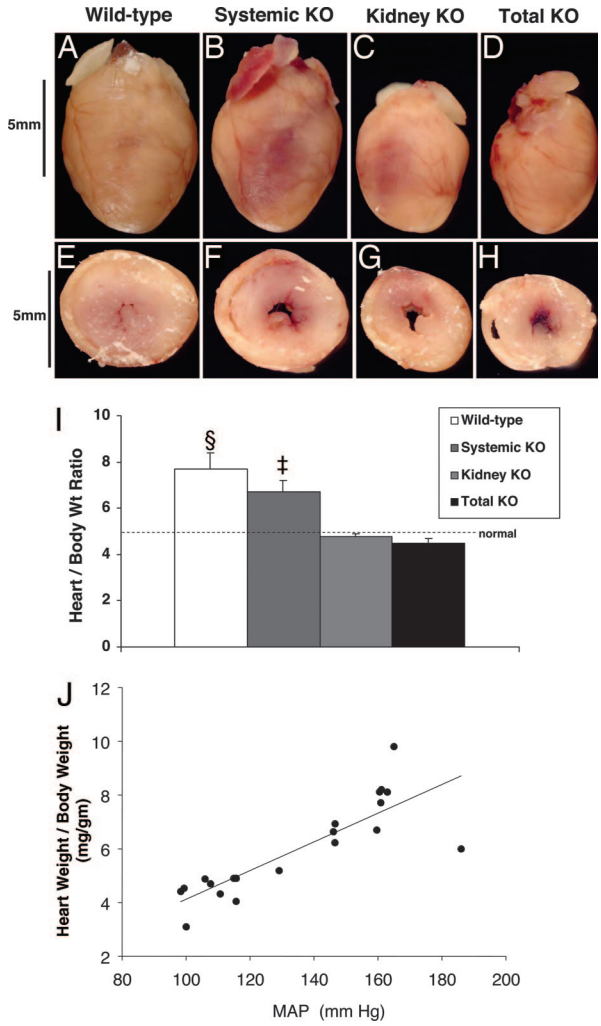

Fig. 2. Cardiac hypertrophy with angiotensin II infusion. (*A–H*) Representative hearts and left ventricular cross-sections after 28 days of Ang II infusion: *A* and *E*, *Wild-type*; *B* and *F*, *Systemic KO*; *C* and *G*, *Kidney KO*; *D* and *H*, *Total KO*. (*I*) Mean heart-to-body weight ratios after 28 days of Ang II infusion. The dashed line represents the mean heart-to-body weight ratio for noninfused *Wild-type* mice established in previous experiments in our laboratory (23). *Wild-type* and *Systemic KO* groups exhibit significant cardiac hypertrophy. ($n \geq 9$ per group; §, $P < 0.002$ vs. *Kidney KO* and $P = 0.0004$ vs. *Total KO*; ‡, $P = 0.003$ vs. *Kidney KO* and $P = 0.0008$ vs. *Total KO*). (*J*) For the entire cohort, there was a significant positive correlation between heart-to-body weight ratio and blood pressure ($R = 0.84$. $P < 0.0001$).

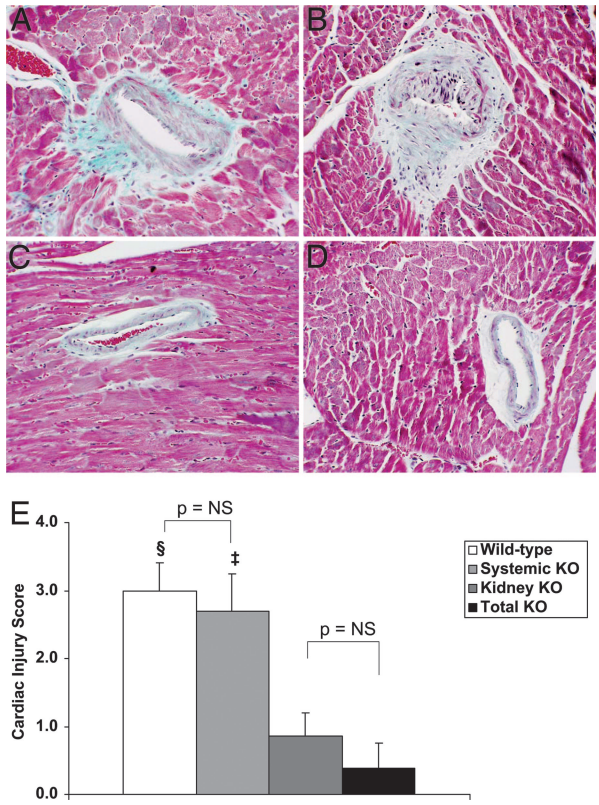

Fig. 3. Cardiac injury after angiotensin II infusion. (*A–D*) Representative photomicrographs of heart sections stained with Masson trichrome. (Magnification: ×20.) Vascular lesions with perivascular infiltrates, medial expansion, and myocyte injury were common in hearts from the *Wild-type* (*A*) and *Systemic KO* (*B*) groups, whereas myocardial and vascular morphology were normal in the *Kidney KO* (*C*) and *Total KO* (*D*) groups. (*E*) Semiquantitative scoring of cardiac pathology (§, $P < 0.002$ vs. *Kidney KO* and $P < 0.0003$ vs. *Total KO*; ‡, $P < 0.02$ vs. *Kidney KO* and $P < 0.004$ vs. *Total KO*).

body weights at baseline and after 5 days of Ang II infusion. Body weights increased significantly in the *Wild-type* (+ 5.9%) and *Systemic KO* (+ 4.2%) groups but did not change significantly in the *Kidney KO* and *Total KO* groups (Fig. 1*C*), exactly paralleling the effects of Ang II on sodium excretion. These data indicate that Ang II causes hypertension by activating AT_1 receptors in the kidney promoting sodium reabsorption. Conversely, when AT_1 receptors in the kidney are absent, sufficient sodium is excreted in the urine to protect against the development of hypertension. These findings suggest that ACE inhibitors and ARBs reduce blood pressure in hypertensive patients by attenuating AT_1 receptor signals in the kidney, thereby facilitating excretion of sodium.

Cardiac Hypertrophy Depends on Blood Pressure Elevation Rather Than Expression of AT_1 Receptors in the Heart. In patients with hypertension, one of the early and most common consequences of chronic hypertension is left ventricular hypertrophy (LVH), and the presence of LVH is associated with significant cardiovascular risk (26, 27). Clinical evidence suggests that the RAS contributes to the development of LVH in hypertension (28–30). In our experiments, *Systemic KO* animals develop severe hypertension but lack AT_{1A} receptors in the heart. Conversely, *Kidney KO* animals are resistant to Ang II-induced hypertension but have a full complement of cardiac AT_{1A} receptors. Thus, we reasoned that we could use our model to separate the effects of elevated blood pressure from direct cellular actions of AT_1 receptors in the development of cardiac hypertrophy.

Accordingly, we compared heart size in the four experimental groups. With Ang II infusion, the *Wild-type* group developed robust cardiac hypertrophy (Fig. 2 *A*, *E*, and *I*), the extent of which was similar to that of previous studies with intact *Wild-type* animals infused with Ang II (23). In the *Systemic KO* group, which develop hypertension but lack AT_{1A} receptors in the heart, the degree of cardiac hypertrophy and increased left ventricular wall thickness was similar to that in the *Wild-type* group (Fig. 2 *B*, *F*, and *I*), indicating that cardiac AT_1 receptors are not necessary for the development of hypertrophy in this model. In contrast, in the *Kidney KO* group, with the normal complement of cardiac AT_{1A} receptors but without hypertension, there was no evidence of hypertrophy after Ang II infusion (Fig. 2 *C*, *G*, and *I*). Moreover, heart sizes and weights in the *Kidney KO* group were virtually identical to those of the *Total KO* group (Fig. 2 *D*, *H*, and *I*). These results suggest that prolonged stimulation of AT_1 receptors in the heart, without blood pressure elevation, is not sufficient to cause cardiac hypertrophy *in vivo*. As shown in Fig. 3*J*, there was a tight correlation between heart

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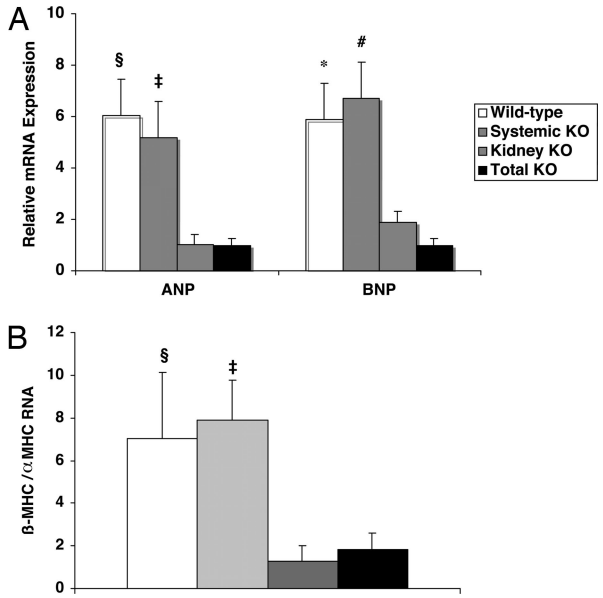

Fig. 4. Cardiac gene expression in mice infused with angiotensin II. (*A*) Expression of ANP and BNP mRNA in hearts ($n \geq 8$ per group; §, $P = 0.003$ vs. *Kidney KO* and $P < 0.002$ vs. *Total KO*; ‡, $P = 0.004$ vs. *Kidney KO* and $P < 0.003$ vs. *Total KO*; *, $P = 0.02$ vs. *Kidney KO* and $P = 0.002$ vs. *Total KO*; #, $P = 0.01$ vs. *Kidney KO* and $P < 0.002$ vs. *Total KO*). (*B*) Ratio of cardiac β-MHC-to-α-MHC mRNA expression in hearts. (§, $P = 0.01$ vs. *Kidney KO* and $P = 0.03$ vs. *Total KO*; ‡, $P = 0.002$ vs. *Kidney KO* and $P = 0.01$ vs. *Total KO*).

weight and blood pressure across all four experimental groups, suggesting that cardiac hypertrophy depends primarily on the level of blood pressure rather than the presence of AT_1 receptors in the heart.

Cardiac Pathology with Ang II Hypertension. We next examined the extent of cardiac histopathology in the four experimental groups. Sections from the *Wild-type* and *Systemic KO* groups showed significant and similar degrees of pathology (Fig. 3 *A* and *B*), including perivascular cellular infiltrates, vessel wall thickening, and myocardial injury. In contrast, vessel wall structure and myocardial architecture were normal in the *Kidney KO* sections (Fig. 3*C*) and virtually indistinguishable from the *Total KO* hearts (Fig. 3*D*). With quantitative scoring (Fig. 3*E*), the two groups with hypertension (*Wild-type* and *Systemic KO*) displayed significant cardiac injury whether or not AT_{1A} receptors were expressed in the heart, whereas cardiac injury was minimal in the *Kidney KO* group despite their normal expression of cardiac AT_{1A} receptors.

Cardiac Gene Expression Patterns During Ang II Hypertension. Although our evaluations of heart weight and histology indicate a dominant effect of blood pressure on cardiac pathology, we considered the possibility that these assessments might not be sufficiently sensitive to detect cellular actions of AT_1 receptors in cardiac myocytes. During hypertrophic cardiac remodeling, gene expression in myocardium undergoes characteristic alterations, including enhanced expression of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), along with recapitulation of fetal gene expression patterns for myosin heavy chains (MHC) characterized by a down-regulation of α-MHC and up-regulation of the β-MHC isoform (31–34). Previous studies have suggested that Ang II may stimulate these processes (35, 36). We therefore examined cardiac mRNA expression for ANP, BNP, α-MHC, and β-MHC after 4 weeks of Ang II infusion. As shown in Fig. 4*A*, levels of mRNA for both ANP and BNP were both dramatically increased in hearts from *Wild-type* animals compared with hearts from the *Total KO* group. Expression was similarly enhanced in the *Systemic KO* group despite their lack of cardiac AT_{1A} receptors. In contrast, up-regulation of ANP or BNP mRNAs was not detected in the *Kidney KO* hearts despite their full complement of cardiac AT_{1A} receptors. A similar pattern was observed in our measurements of β-MHC and α-MHC mRNA levels. As depicted in Fig. 4*B*, the ratio of β-MHC/α-MHC expression was increased by nearly 4-fold in the *Wild-type* and *Systemic KO* groups compared with the *Kidney KO* and *Total KO* groups.

Discussion

A major role for the RAS in promoting hypertension and its associated end-organ damage has long been recognized (37–39). These actions are mediated primarily by AT_1 receptors (30, 40). Expression of AT_1 receptors in key sites involved in regulation of peripheral vascular resistance, sodium balance, and activity of the sympathetic nervous system is also consistent with its critical role in blood pressure homeostasis. Because pharmacological antagonists and conventional gene knockouts produce global inhibition of AT_1 receptors, it has been difficult to discriminate clearly which of these specific tissue sites mediate the pathogenic actions of AT_1 receptors in hypertension. Accordingly, we developed an experimental model of kidney cross-transplantation in the mouse that allows for separation of AT_1 receptor pools in the kidney from those in other tissues.

A major finding in our study is that AT_1 receptors in the kidney are primarily responsible for the actions of Ang II to cause hypertension. This is clearly illustrated in the *Systemic KO* animals, where the presence of AT_{1A} receptors *only* in the kidney is sufficient to recapitulate the phenotype of hypertension with Ang II infusion. Conversely, in the *Kidney KO* group, the absence of AT_1 receptors from the kidney alone is sufficient to protect these animals from Ang II-dependent hypertension, despite the expression of AT_{1A} receptors in a number of other key areas that potentially impact blood pressure homeostasis, including the brain, the heart, the peripheral vasculature, and the adrenal gland. The mechanism for the distinct blood pressure responses in the two groups appears to be related to differences in renal sodium handling. Ang II infusion is associated with reduced renal sodium excretion and weight gain in the *Systemic KO* group, whereas the absence of renal AT_1 receptors in the *Kidney KOs* is associated with enhanced natriuresis, no change in weight, and resistance to hypertension. It is important that these effects on blood pressure and sodium excretion are determined by direct actions of AT_1 receptors within the kidney, independent of any contribution of Ang II-dependent aldosterone release.

Within the kidney, AT_1 receptors are expressed on epithelial cells throughout the nephron, in the glomerulus, and on the renal vasculature (41, 42). In the proximal tubule, AT_1 receptors promote sodium reabsorption by coordinately stimulating the sodium-proton antiporter on the luminal membrane along with the sodium-potassium ATPase on the basolateral surface (43, 44). AT_1 receptors also affect sodium reabsorption in distal nephron segments, including the thick ascending limb, distal tubule, and collecting duct (45–48). In the collecting duct, for example, Bell and associates have shown that Ang II directly stimulates epithelial sodium channel activity through an AT_1 receptor-dependent mechanism (49). AT_1 receptors expressed in the renal vasculature also have important regulatory effects on sodium handling (50). Renal vasoconstriction caused by Ang II, which we have previously shown to be primarily mediated by AT_{1A} receptors (51), reduces medullary blood flow, blunting the kidney's excretory capacity for sodium (52, 53). Our study suggests that the primary mechanism of action of ACE inhibitors and ARBs to reduce blood pressure in hypertensive patients is attenuation of AT_1 receptor signals at one or more of these key sites in the kidney.

In previous work focused on normal blood pressure homeostasis, we found that AT_1 receptor actions in the kidney and extrarenal

tissues made virtually equivalent contributions to preventing hypotension and supporting normal blood pressure (21). Thus, when circulatory volumes are threatened, the full range of AT_1 receptor actions at key tissue sites is activated and apparently necessary to protect against circulatory collapse. On the other hand, their relative contributions appear to be quite different in hypertension where the population of AT_1 receptors in the kidney assumes a preeminent role. In this regard, Guyton hypothesized that the substantial capacity for sodium excretion by the kidney provides a compensatory system of virtually infinite gain to oppose processes, including increases in peripheral vascular resistance, which would tend to increase blood pressure (2). It follows that defects in renal excretory function would be a prerequisite, therefore, for sustaining a chronic increase in intra-arterial pressure. Our current findings confirm the key role of altered renal sodium handling in Ang II-dependent hypertension and are completely consistent with Guyton's hypothesis.

There was a statistically significant increase in blood pressure in the *Kidney KO* group coinciding with the initiation of the angiotensin II infusion. This increase in blood pressure rapidly peaked on Day 2 and then gradually returned toward baseline. We speculate that this early change in blood pressure was due to vasoconstriction mediated by AT_1 receptors in the peripheral vasculature. Its rapid attenuation was likely due to the accompanying natriuresis. This pattern illustrates the relatively modest contribution of systemic vasoconstriction, in isolation, to the development of Ang II-dependent hypertension. Even at their peak, blood pressures in the *Kidney KOs* remained substantially lower than those in the *Wild-type* group. Furthermore, blood pressures in this group were consistently and substantially reduced compared with those of the *Systemic KOs*. Thus, chronic systemic vasoconstriction driven by Ang II has a very limited capacity to cause sustained hypertension.

The pattern in the *Systemic KO* group was quite different. Blood pressure increased progressively during the first 2 weeks, although the rate of rise was delayed compared with that of the *Wild-type* group. We suggest that this early difference reflects the absence of the transient vasoconstrictor response seen in the *Kidney KOs*. With time, however, pressures in the *Systemic KOs* eventually reach the level of the *Wild-type* group, indicating that AT_1 receptor actions in the kidney are sufficient to generate the full hypertension phenotype. Moreover, as discussed below, the similar extent of cardiac hypertrophy seen in the *Wild-type* and *Systemic KO* groups, and its complete absence in the *Kidney KO* group, suggests that the late, sustained phase of hypertension provides the major stimulus for end-organ injury in this model.

A major goal of hypertension treatment is to prevent or ameliorate injury to key target organs, including the heart, kidney, and brain. One of the most prevalent manifestations of end-organ damage in hypertension is the development of LVH (26), and its presence confers substantial cardiovascular risk (26, 27). Although pressure load from elevated blood pressure clearly contributes to LVH, several lines of evidence suggest that activation of the RAS also plays a role. For example, Ang II, acting through AT_1 receptors, stimulates hypertrophy of cardiac myocytes in culture (54, 55). In addition, clinical studies have demonstrated actions of ACE inhibitors and ARBs to cause regression of LVH more effectively than other classes of antihypertensive agents with similar levels of blood pressure control (28–30).

Although distinguishing the relative contributions of hypertension and cardiac AT_1 receptor activation to the development of LVH *in vivo* has been difficult, we reasoned that our model might be useful for this purpose. The extent of blood pressure elevation was very similar in the *Wild-type* and *Systemic KO* groups, but they differ in their expression of AT_{1A} receptors in the heart. Despite lacking cardiac AT_{1A} receptors, the *Systemic KO* group developed robust LVH with heart weights that were not significantly different from those of the *Wild-type* group. By contrast, *Kidney KOs* with a normal complement of cardiac AT_{1A} receptors do not develop significant hypertension and likewise have no appreciable change in their heart weights. Within the entire cohort, there was a tight linear correlation between heart weight and blood pressure irrespective of the presence or absence cardiac AT_{1A} receptors. Thus, in a simple model of Ang II-dependent hypertension, the severity of cardiac hypertrophy was exclusively dependent on blood pressure. We found no evidence for a contribution of direct actions of AT_1 receptors in the heart to promote LVH. Although our data are clear-cut, they do not necessarily obviate the results of well-designed prospective clinical trials demonstrating beneficial effects of ACE inhibitors or ARBs on regression of LVH (29, 30). Rather, they suggest that the benefits of these agents in LVH are not a consequence of blocking cellular actions of Ang II in the heart, but may be due to differences in the degree or pattern of blood pressure control that was achieved (56).

Direct effects of AT_1 receptor activation to promote inflammation, fibrosis, and vascular damage have been implicated as pathways for facilitating end-organ damage in hypertension (22, 24, 57–61). In our studies, we found that Ang II infusion caused cardiac fibrosis and vascular pathology in the *Wild-type* and *Systemic KO* groups. On the other hand, hearts in the *Kidney KOs* appeared virtually normal, indicating that vascular injury and fibrosis in the heart were also consequences of elevated blood pressure rather than local actions of cardiac AT_1 receptors. Cardiac hypertrophy is associated with characteristic alterations in gene expression, including up-regulation of ANP and BNP (31, 32), and recapitulation of fetal patterns for expression of myosin heavy chains (33, 34). It has been suggested that activation of AT_1 receptors in cardiomyocytes may be sufficient to trigger this transcription profile (34, 35). However, as we observed with cardiac hypertrophy and pathology, activation of AT_1 receptors in the heart is not responsible for characteristic gene expression patterns associated with Ang II infusion; these are also triggered by elevated blood pressure.

In summary, these studies provide incontrovertible evidence that angiotensin II causes hypertension through actions of AT_1 receptors expressed in the kidney that reduce urinary sodium excretion. This is a direct effect of AT_1 receptors in the kidney that does not involve or require angiotensin II-mediated aldosterone responses in the adrenal gland. Our findings suggest that the mechanism of antihypertensive actions of ACE inhibitors and ARBs involves attenuation of the renal actions of angiotensin II. Furthermore, these experiments indicate that the major mechanism of protection from cardiac hypertrophy afforded by these agents is related to blood pressure control rather than inhibition of AT_1 receptor actions in the heart.

Materials and Methods

Animals. $(129 \times C57BL/6)F_1$ mice lacking AT_{1A} receptors for Ang II were generated as described in ref. 14. Animals were bred and maintained in the animal facility of the Durham Veterans Affairs Medical Center under National Institutes of Health guidelines. These studies used 2- to 4-month-old male mice.

Mouse Kidney Transplantation. Vascularized kidney transplants were performed in mice as described in ref. 21. The donor kidney, ureter, and bladder were harvested *en bloc*, including the renal artery with a small aortic cuff and the renal vein with a small vena caval cuff. These vascular cuffs were anastomosed to the recipient abdominal aorta and vena cava, respectively, below the level of the native renal vessels. Donor and recipient bladders were attached dome to dome. The right native kidney was removed at the time of transplant, and the left native kidney was removed through a flank incision 1–3 days later. The adrenal glands and their blood supply were preserved intact. Each group consisted of at least seven animals.

Telemetry Probe Implantation Procedure. The components of the radiotelemetry system (Transoma Medical, St. Paul, MN), includ-

ing the mouse blood pressure telemetry device (TA11PA-C20), are described in ref. 62. Six to 8 days after the kidney transplantation procedure, the pressure catheter was implanted in the left carotid artery as described in ref. 63.

Telemetric Blood Pressure Analysis. Data were collected, stored, and analyzed by using Dataquest A.R.T. software (Transoma Medical). Blood pressures were measured on unanesthetized, unrestrained animals beginning 7 days after the catheter implantation when the mice had reestablished normal circadian rhythms (63). Telemetry data were collected continuously with sampling every 6 min for 10-s intervals (62).

Experimental Protocol. Baseline blood pressure measurements were determined on 3 consecutive days while the animals ingested a normal diet containing 0.4% sodium chloride. After these baseline recordings, an osmotic minipump (Alzet Model 2004; DURECT) infusing Ang II (Sigma, St. Louis, MO) at a rate of 1,000 ng/kg/min was implanted s.c. as described in ref. 23, and blood pressure measurements continued for 21 days.

Metabolic Balance Studies. One week after transplantation, the animals were placed in specially designed metabolic cages (23). The mice were fed 10 gm/day gelled 0.25% NaCl diet that contained all nutrients and water (Nutra-gel; Bio-Serv, Frenchtown, NJ). After 1 week of baseline collections, the animals were implanted with osmotic minipumps infusing Ang II as described above and were returned to the metabolic cage for 5 more days. Urinary sodium content was determined by using an IL943 Automatic Flame photometer per the manufacturer's instructions (Instrumentation Laboratory, Lexington, MA).

Histopathologic Analysis. After 28 days of Ang II infusion, hearts were harvested, weighed, fixed in formalin, sectioned, and stained with Masson trichrome. All of the tissues were examined by a pathologist (P.R.) without knowledge of genotypes. Pathology was graded based on the presence and severity of component abnormalities, including cellular infiltrate, myocardial cell injury, vessel wall thickening, and fibrosis. Grading for each component was performed by using a semiquantitative scale where 0 was normal and 1–4+ represented mild through severe abnormalities. The total cardiac injury score for each heart was a summation of the component injury scores.

Quantification of Cardiac mRNA Expression. Hearts were harvested, and total RNA was isolated by using an RNeasy mini kit per the manufacturer's instructions (Qiagen, Valencia, CA). The gene expression levels of ANP, BNP, α-MHC, and β-MHC in cardiac tissue were determined by real-time quantitative RT-PCR as reported in ref. 64.

Statistical Analysis. The values for each parameter within a group are expressed as the mean ± SEM. For comparisons between groups with normally distributed data, statistical significance was assessed by using ANOVA followed by an unpaired t test; within groups, a paired t test was used. For nonparametric comparisons, the Mann–Whitney U test was used between groups, and the Wilcoxon signed rank test was used within groups.

We acknowledge outstanding administrative support from Ms. Norma Barrow and technical support from Mr. Chris Best. This work was supported by National Institutes of Health Grants HL49277 and HL56122 and by funding from the Medical Research Service of the Department of Veterans Affairs.

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